ENDOTHELIAL BARRIER AND METABOLISM:

NEW KIDS ON THE BLOCK REGULATING BONE MARROW VASCULAR NICHES.

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ABSTRACT

The vasculature of the bone marrow remains poorly characterized, although crucial to maintain hematopoiesis and retain stem cells in a quiescent state. A recent study by Itkin et al. in *Nature* reports how vascular barrier integrity and endothelial cell metabolism regulate hematopoietic stem cell quiescence and leukocyte trafficking.

MAIN TEXT

The best-known function of blood vessels, and of their lining endothelial cells (ECs), is to supply oxygen and nutrients to tissues, but blood vessels are also conduits for hematopoietic cells during immune surveillance. Indeed, in the bone marrow (BM) (a prime site of hematopoiesis), ECs control trafficking of leukocytes to and from the marrow. BM blood vessels also have perfusion-independent functions, such as creating a vascular niche for hematopoietic stem and progenitor cells (HSPCs). An outstanding question was whether leukocyte trafficking and hematopoietic stem cell (HSC) preservation occur at the same vascular site. Earlier studies identified sinusoidal vessels and arterioles as vascular niches for HSPCs (Kiel et al., 2005; Kunisaki et al., 2013; Ludin et al., 2014). Using improved real-time imaging techniques, Itkin et al. (2016) now report that arterial ECs in the BM (aBMECs) create an endosteal vascular niche for non-active quiescent HSCs, while sinusoidal ECs (sBMECs) constitute an exclusive site for leukocyte trafficking and HSPC activation (Figure). Hypothesizing that reactive oxygen species (ROS) regulate HSC quiescence, the authors made the fascinating discovery that only ROS^{low} HSPCs, believed to contain the most primitive long-term repopulating HSCs (Jang and Sharkis, 2007), were detected at aBMEC niches, while ROS^{high} HSPCs, known to be less primitive, were detected at sBMEC niches. This was unexpected, as aBMECs are exposed to higher oxygen levels and thus prone to higher ROS production, which these ECs seemingly counteract by relying on glycolysis.

The Itkin et al. (2016) study is also special, as they define for the first time an important role for vascular barrier integrity in hematopoiesis. Indeed, aBMECs have a less permeable vascular barrier than sBMECs, which protects perivascular cells against access to blood-borne ROS-inducing agents. Hence, BMEC barrier disruption promotes leukocyte trafficking at the expense of stem cell maintenance, through an increase in ROS levels in HPSCs. Manipulating the mechanical barrier and metabolism of BM vessels may have implications for HSC transplantation and mobilization strategies.

While ECs line blood vessels in all tissues, they vary in structure and function, even within a single tissue, in order to fulfill vascular bed-specific needs (Aird, 2012). Itkin et al. (2016) show that ECs in different BM vascular beds exhibit prominent differences. Arterioles in the endosteal zone sustain HSC quiescence, while sinusoids are an exclusive site of leukocyte trafficking. Notably, aBMECs have a different anatomical, hemodynamic, molecular, and metabolic signature as compared to sBMECs. Indeed, they line small arteries or arterioles with higher flow and shear rates, are located primarily in endosteal regions, are enwrapped by mural pericytes (arteries) or HSC-supportive mesenchymal stromal precursor cells (arterioles), and express nestin and Sca-1. These vessels are less permeable due to higher expression of junctional proteins like VE-Cadherin. Metabolically, even though they are exposed to higher oxygen levels and thus are expected to rely more on oxidative metabolism (which can produce ROS as byproducts), they have lower ROS levels, presumably because they rely more on glycolysis than sBMECs. Conversely, more permeable fenestrated sinusoids induce higher ROS in their surroundings, and have slower blood flow in their wider lumen (offering a greater surface for cell exchange), hence serving as an ideal site for cell trafficking.

These distinct identities also confer distinct functional destinies to aBMECs *versus* sBMECs. Indeed, quiescent HSCs, which are averse to high ROS conditions, thrive best in the less permeable, ROS^{low} aBMEC niche, while trafficking leukocytes take profit of the more permeable sBMEC site, where ROS^{high} conditions promote HSPC mobilization and differentiation (Itkin et al., 2016; Ludin et

al., 2014). Interestingly, if the metabolic (ROS) state was ignored, the physical distribution of HSPCs was random among distinct BM regions (Itkin et al., 2016). The Itkin et al. (2016) study thus provides another layer of complexity while at the same time simplifying the existing model that depicts both sinusoidal and arteriolar vessels as vascular niches (Kiel et al., 2005; Kunisaki et al., 2013; Ludin et al., 2014). Earlier studies argued that ECs and perivascular cells in these niches produce signals that control HSC maintenance, expansion and lineage-specific differentiation (Kunisaki et al., 2013; Ludin et al., 2014). Itkin et al. (2016) now argue a clear separation of HSC quiescence and leukocyte trafficking at distinct vascular sites. While this is now shown for the first time, an earlier study already provided suggestive evidence for functionally separate BMEC sites. Indeed, computational modeling argued that the association of HSPCs with sBMECs was rather random, whereas the association with arterioles, occupying only 1% of the BM volume, was highly significant (Kunisaki et al., 2013).

The Itkin et al. (2016) study discovered a previously unrecognized role for vascular permeability in modulating HSC quiescence and leukocyte trafficking. Using pharmacological and genetic approaches, Itkin et al. show that manipulating the BMEC barrier affects hematopoiesis. Rendering the barrier more leaky compromised HSC quiescence at the aBMEC site, while promoting HSPC mobilization and differentiation, and increasing bidirectional trafficking at the sBMEC site. Barrier integrity of BMECs is linked to ROS levels in HSPCs, as ROS production seems to be a direct response to increased leakiness and exposure to blood plasma, which can penetrate the BM more easily via fenestrated sinusoids (Itkin et al., 2016). Of note, ROS may not only be the result of barrier permeability, but ROS may also causally promote EC barrier disruption (Eelen et al., 2015).

Quiescent HSCs attempt to keep ROS levels low (Ludin et al., 2014). In agreement, HSCs have fewer mitochondria than hematopoietic progenitors (and thus generate less ROS in oxidative metabolism), and their quiescent state depends on higher rates of glycolysis (Takubo et al., 2013).

Since oxygen levels in the peri-arteriolar BM regions are 2-fold higher than in peri-sinusoidal regions (Spencer et al., 2014), glycolysis in HSCs may enable them to minimize ROS exposure.

The Itkin et al. study also raises questions. Itkin et al. suggest that aBMECs rely on glycolysis to create a ROS^{low} environment for HSCs. A more in depth metabolic characterization of BMEC subtypes will be advantageous to confirm this hypothesis. Also, do aBMECs and sBMECs differ in other metabolic aspects, important for HSC quiescence *versus* cell trafficking? Which metabolism pathways ECs are geared to use, can determine their phenotype (De Bock et al., 2013). Further characterization of BMEC metabolism might yield interesting novel insights.

The Itkin et al. study may also have implications for stem cell therapy, requiring mobilization of HSCs while maintaining repopulating abilities. An exciting question is whether induction of hyperpermeability, to decrease retention of HSCs in their arteriolar niche, can improve collection efficacy. Conversely, tightening the BMEC barrier may be beneficial to promote BM lodging and engraftment of transplanted HSCs. Another intriguing question is whether inhibiting oxidative metabolism of BMECs and HSCs, thus possibly rendering them more glycolytic, would be beneficial. Finding an answer to these potentially clinically relevant questions will be an exciting journey.

FIGURE LEGEND: Vascular BM niches with distinct vascular barrier and metabolism features, which can be manipulated therapeutically.

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